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Oxygen-derived Species: Their Relation to Human Disease and Environmental Stress

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Free radicals and other reactive oxygen species (ROS) are constantly formed in the human body, often for useful metabolic purposes. Antioxidant defenses protect against them, but these defenses are not completely adequate, and systems that repair damage by ROS are also necessary. Mild oxidative stress often induces antioxidant defense enzymes, but severe stress can cause oxidative damage to lipids, proteins, and DNA within cells, leading to such events as DNA strand breakage and disruption of calcium ion metabolism. Oxidative stress can result from exposure to toxic agents, and by the process of tissue injury itself. Ozone, oxides of nitrogen, and cigarette smoke can cause oxidative damage; but the molecular targets that they damage may not be the same. — *Environ Health Perspect* 102(Suppl 10):5–12 (1994)

Key words: free radical, oxygen radical, superoxide, hydroxyl, hydrogen peroxide, oxidative stress, transition metals, ozone, nitrogen dioxide, cigarette smoke

Introduction

There is considerable current interest in the role of free radicals, oxygen radicals, and oxidative stress as mediators of tissue injury in human disease and of the effects of air pollutants, such as ozone (O₃), nitrogen dioxide (NO₂), and tobacco smoke. This article explains the meaning of these terms and summarizes current knowledge of the roles they play in these various situations.

What Is a Radical?

In the structure of atoms and molecules, electrons usually associate in pairs, each pair moving within a defined region of space (an atomic or molecular *orbital*). One electron in each pair has a spin quantum number of +1/2, the other –1/2. A free radical is any species capable of independent existence (hence the term “free”) that contains one or more unpaired electrons, an unpaired electron being one that is alone in an orbital (1). The simplest free radical is a hydrogen atom, with one proton and a single electron. Table 1 gives some examples of other free radicals. Note that the gases nitric oxide (NO•) and nitrogen dioxide (NO₂•) are free radicals, whereas ozone (O₃) is not—no unpaired electrons are present. The spectroscopic

technique of electron spin resonance is often used to measure free radicals; it records the energy changes that occur as unpaired electrons align in response to a magnetic field. A superscript dot (•) is used to denote free radical species.

Oxygen—A Free Radical and Environmental Toxin

When living organisms first appeared on the earth, they did so under an atmosphere containing very little O₂, i.e., they were essentially anaerobes. Anaerobic microorganisms still survive to this day, but their growth is inhibited and they can often be killed by exposure to 21% O₂, the current atmospheric level. As the O₂ content of the atmosphere rose (due to the evolution of organisms with photosynthetic water-splitting capacity) many primitive organisms probably died. Present-day anaerobes are presumably the descendants of those primitive organisms that followed the evolutionary path of “adapting” to rising atmospheric O₂ levels by restricting themselves to environments that O₂ did not penetrate. However, other organisms began the evolutionary process of evolving antioxidant defense systems to protect against O₂ toxicity. In retrospect, this was a fruitful path to follow since organisms that tolerated the presence of O₂ could also evolve to use it for metabolic transformations (involving enzymes such as oxidases and oxygenases) and for efficient energy production (by using electron transport chains with O₂ as the terminal electron acceptor, such as the mitochondrial oxidative phosphorylation system). Hence, O₂ was probably the first environ-

mental air pollutant to appear in large quantities on the planet.

However, even present-day aerobes suffer oxidative damage if they are exposed to O₂ at concentrations greater than 21% (2). Oxygen toxicity has been demonstrated in plants, animals and microorganisms. For example, exposure of adult humans to pure O₂ at 1 atm pressure for as little as 6 hr causes chest soreness, cough, and sore throat in some subjects; and longer periods of exposure lead to lung damage. The incidence of ocular damage in babies known as retrolental fibroplasia (“formation of fibrous tissue behind the lens”) increased abruptly in the early 1940s among babies born prematurely and led to many cases of blindness. Not until 1954 was it realized that retrolental fibroplasia is associated with the use of high O₂ concentrations in incubators for premature babies. More careful control of O₂ concentrations (continuous transcutaneous O₂ monitoring, with supplementary O₂ given only where necessary) and administration of α -tocopherol have decreased its incidence, but the problem has not disappeared, since many premature infants need continuous high O₂ to survive at all (3).

The damaging effects of elevated O₂ on aerobes vary considerably with the organism studied, age, physiologic state, and diet; and different tissues are affected in different ways. Thus, cold-blooded animals such as turtles and crocodiles are relatively resistant to O₂ at low environmental temperatures, but become more sensitive at higher temperatures. Neonatal rats resist lung damage in an atmosphere of 100% O₂ far more effectively than do adult rats (2).

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Table 1. Examples of free radicals.

Name	Formula	Comments
Hydrogen atom	H•	The simplest free radical known.
Trichloromethyl	CCl ₃ •	A carbon-centered radical (i.e., the unpaired electron resides on carbon). CCl ₃ • is formed during metabolism of CCl ₄ in the liver and contributes to the toxic effects of this solvent (7).
Superoxide	O ₂ • ⁻	An oxygen-centered radical.
Hydroxyl	OH•	An oxygen-centered radical. The most highly reactive oxygen radical known.
Thiyl	RS•	General name for a group of radicals with an unpaired electron residing on sulfur.
Peroxy, alkoxy	RO ₂ •, RO•	Oxygen-centered radicals formed during the breakdown of organic peroxides.
Oxides of nitrogen	NO•, NO ₂ •	Both are free radicals. NO• is formed <i>in vivo</i> from the amino acid L-arginine (8). NO ₂ • is made when NO• reacts with O ₂ and is found in polluted air and smoke from burning organic materials, e.g., cigarette smoke.

The earliest suggestion made to explain O₂ toxicity was that O₂ is a direct inhibitor of enzymes, thereby interfering with metabolism. However, very few targets of direct damage by O₂ have been identified in aerobes. In 1954, Gerschman et al. (4) proposed that the damaging effects of O₂ could be attributed to the formation of oxygen radicals. This hypothesis was popularized and converted into the superoxide theory of O₂ toxicity following the discovery of superoxide dismutase (SOD) enzymes by McCord and Fridovich (5). In its simplest form, this theory states that O₂ toxicity is due to excess formation of superoxide radical (O₂•⁻), the one electron reduction product of O₂, and that the SOD enzymes are important antioxidant defenses because they remove O₂•⁻. Ironically, with all the fuss made about oxygen radicals, it must be realized that the diatomic oxygen molecule is itself a free radical, containing two unpaired electrons (1). Fortunately, the electronic arrangement in O₂ renders this molecule unreactive despite its free radical nature (1).

Reactive Oxygen Species *in Vivo*

Reactive oxygen species (ROS) is a collective term used by biologists to include not only oxygen radicals (O₂•⁻ and hydroxyl radical, OH•) but also some derivatives of O₂ that do not contain unpaired electrons, such as hydrogen peroxide (H₂O₂), singlet O₂ (¹Δg), and hypochlorous acid (HOCl)*. Reactive is of course a relative term: O₂•⁻ is more reactive than O₂, but neither O₂•⁻ nor H₂O₂ in aqueous solution is anywhere near as reactive as OH• (1).

All organisms suffer some exposure to OH•, because it is generated *in vivo* by homolytic fission of O–H bonds in water, driven by our continuous exposure to background ionizing radiation (6). Hydroxyl radical is so reactive with all biological molecules that it is impossible to evolve a specific scavenger of it—almost everything in living organisms reacts with OH• with second-order rate constants of 10⁹ to 10¹⁰ M⁻¹sec⁻¹ (essentially, if OH• contacts the compound, reaction occurs). Damage caused by OH•, once this radical has been formed, is probably unavoidable and is dealt with by repair processes (Table 2).

It is now well established (1,5,18–20) that O₂•⁻ and H₂O₂ are produced in aerobes,

although the precise amounts generated and the steady-state concentrations achieved are still uncertain. Generation of these species occurs by two types of processes described below.

“Accidental” Generation. This encompasses such mechanisms as “leakage” of electrons onto O₂ from mitochondrial electron transport chains, microsomal cytochromes P450 and their electron donating enzymes, and other systems (1,5,20). It also includes so-called autooxidation reactions in which compounds such as catecholamines, ascorbic acid, and reduced flavins are alleged to react directly with O₂ to form O₂•⁻ (5). In fact, such autooxidations are usually catalyzed by transition metal ions (1).

Deliberate Synthesis. The classic example of deliberate metabolic generation of ROS for useful purposes is the production of O₂•⁻, HOCl, and H₂O₂ by activated phagocytes (21). Hydrogen peroxide is additionally generated *in vivo* by several oxidase enzymes, such as glycolate oxidase, xanthine oxidase, and D-amino acid oxidase (18,22). Evidence is accumulating that O₂•⁻ is also produced by several cell types other than phagocytes, including lymphocytes (23), fibroblasts (24,25), and vascular endothelial cells (26–28). Such O₂•⁻ might be involved in intercellular signalling and could serve important biologic functions, although more information is needed.

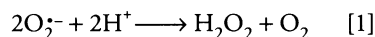
Table 2. Repair of oxidative damage.

Substrate of damage	Repair system	Representative recent references
DNA All components of DNA can be attacked by OH•, whereas singlet O ₂ attacks guanine preferentially. H ₂ O ₂ and O ₂ • ⁻ do not attack DNA (9).	A wide range of enzymes exist that recognize abnormalities in DNA and remove them by excision, resynthesis, and rejoining of DNA strand (10).	(9,10)
Proteins Many ROS can oxidize –SH groups. OH• attacks many amino acid residues (11). Proteins often bind transition metal ions, making them a target of attack by site-specific OH• generation (1,11).	Oxidized methionine residues may be repaired by methionine sulfoxide reductase. Other damaged proteins may be recognized and preferentially destroyed by cellular proteases.	(12,13)
Lipids Some ROS (not including O ₂ • ⁻ or H ₂ O ₂) can initiate lipid peroxidation (14).	Chain-breaking antioxidants (especially α-tocopherol) remove chain-propagating peroxy radicals. Phospholipid hydroperoxide glutathione peroxidase can remove peroxides from membranes, as can some phospholipases. Normal membrane turnover can release damaged lipids.	(15–17)

*HOCl could equally well be regarded as a “reactive chlorine species.”

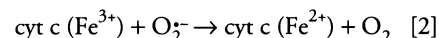
Generation of O_2^- , HOCl, and H_2O_2 by phagocytes is known to play an important part in the killing of several bacterial and fungal strains (21). Some other metabolic roles for H_2O_2 have been proposed (29–33). For example, H_2O_2 is used by the enzyme thyroid peroxidase to help make thyroid hormones (30). H_2O_2 or products derived from it can displace the inhibitory subunit from the cytoplasmic gene transcription factor NF-KB. The active factor migrates to the nucleus and activates genes by binding to specific DNA sequences in enhancer and promoter elements. Thus, H_2O_2 can induce expression of genes controlled by NF-KB. This is of particular interest because NF-KB can induce the expression of genes of the provirus HIV-1, the major cause of acquired immunodeficiency syndrome (33). H_2O_2 , a nonradical, resembles water in its molecular structure and is very diffusible within and between cells.

Much O_2^- generated *in vivo* probably undergoes a dismutation reaction to give H_2O_2 , as represented by the overall equation

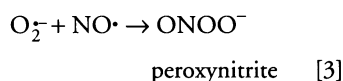


Toxicity of Superoxide and Hydrogen Peroxide

Experimental data show clearly that removal of O_2^- and H_2O_2 by antioxidant defense systems is essential for healthy aerobic life (1,5,18,34). Why is this? In organic media, O_2^- can be very reactive but in aqueous media it is not, mainly acting as a moderate reducing agent, e.g., the reduction of cytochrome c



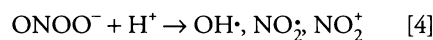
However, O_2^- can react with some targets. In particular, O_2^- reacts fast with nitric oxide in a radical addition reaction (35).



$NO \cdot$ is known to be produced *in vivo* by vascular endothelial cells, by some cells in the brain, and by phagocytes (8). $NO \cdot$ performs useful physiologic functions, such as regulation of vascular smooth muscle tone (hence controlling blood pressure) and neurotransmitter action (8). Since $NO \cdot$ acts upon smooth muscle cells in blood vessel walls to produce relaxation,

then O_2^- , by removing $NO \cdot$ (Equation 3), can act as a vasoconstrictor, and this might have deleterious effects in some clinical situations (36,37).

Considerable debate continues in the literature as to whether or not the interaction of O_2^- and NO (Equation 3) is damaging to cells (38). Peroxynitrite might be directly toxic to cells (38,39). It might also decompose to form a range of toxic products, including some $OH \cdot$ (38,40).

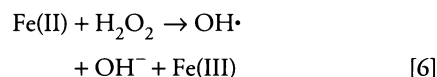
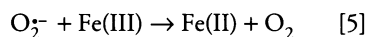


Note that Equation 4 also produces the toxic free radical gas nitrogen dioxide. However, the physiologic significance of these reactions is still uncertain, since some experiments suggest that $NO \cdot$ may protect against oxidative damage even when O_2^- is being generated (41,42).

Superoxide has also been shown to be capable of inactivating several bacterial enzymes, such as *Escherichia coli* dihydroxyacid dehydratase, aconitase, and 6-phosphogluconate dehydratase (5,20,43). It appears to attack iron-sulfur clusters at the enzyme active sites. Whether such reactions of O_2^- occur in mammals is uncertain, although in isolated submitochondrial particles, O_2^- has been claimed to inactivate the NADH dehydrogenase complex of the mitochondrial electron transport chain (44). The protonated form of O_2^- hydroperoxyl radical (HO_2), is much more reactive than O_2^- *in vitro*. For example, HO_2 can initiate peroxidation of polyunsaturated fatty acids and decompose lipid hydroperoxides, which O_2^- cannot (45,46). However, there is no direct evidence that HO_2 exerts damaging effects *in vivo*. The pK_a of HO_2 is about 4.8, so only a small fraction of O_2^- is protonated at physiologic pH values.

H_2O_2 at low micromolar levels also appears poorly reactive (1). However, higher levels of H_2O_2 can attack several cellular energy-producing systems, e.g., by inactivating the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (47).

It is usually thought that most or all of the toxicity of O_2^- and H_2O_2 involves their conversion into $OH \cdot$ (1,48). Several mechanisms have been proposed to explain this. The most recent is the interaction of O_2^- and $NO \cdot$ (Equations 3,4). An earlier proposal (5,48) was the superoxide-driven Fenton reaction



Although there has been repeated controversy in the literature as to whether $OH \cdot$ is formed in such reactions at physiologic pH, the evidence is now overwhelming (49–51). Copper can also catalyze $OH \cdot$ formation from H_2O_2 (52).

Iron and copper (and other transition metal ions) in chemical forms that can decompose H_2O_2 to $OH \cdot$ are in very short supply *in vivo*: organisms are very careful to ensure that as much iron and copper as possible are kept safely bound to transport or storage proteins. Indeed, the “sequestration” of metal ions into forms that will not catalyze free radical reactions is an important antioxidant defense mechanism (1,29,53). Sequestration of iron and copper ions deters the growth of many bacterial strains in human blood plasma (54): it also ensures that plasma will not convert O_2^- and H_2O_2 into $OH \cdot$ (29,53). Prevention of $OH \cdot$ formation may allow small quantities of O_2^- and H_2O_2 released into the extracellular environment (e.g., from endothelial cells, lymphocytes, and phagocytes) to perform useful metabolic roles, such as intercellular signaling, rather than causing damage (29).

In any case, any transition metal ions that do become available to catalyze free radical reactions *in vivo* will not exist in the “free” state for very long. Thus, if iron ions are liberated, they must bind to a biological molecule or else eventually precipitate out of solution as ferric hydroxides, oxyhydroxides, and phosphates. If metal ions bound to a biological molecule react with O_2^- and H_2O_2 (Equations 5,6), $OH \cdot$ will be formed upon the molecule. This $OH \cdot$ -mediated damage is said to be “site specific” (55). Binding of metal ions to a target means that any $OH \cdot$ generated will tend to react with that target rather than with any added scavenger, and the $OH \cdot$ will be very difficult to intercept by $OH \cdot$ scavengers.

It follows that a major determinant of the nature of the damage done by excess generation of ROS *in vivo* is the availability and location of metal ion catalysts of $OH \cdot$ radical formation (1,29). If, for example, “catalytic” iron or copper ions are bound to DNA in one cell type and to membrane lipids in another, then excessive formation of H_2O_2 and O_2^- will, in the first case, damage the DNA and in the other could initiate lipid peroxidation. Evidence for $OH \cdot$ formation in the nucleus of cells treated with H_2O_2 has been

obtained, presumably involving metal ions bound upon, or very close to DNA (56,57).

E. coli mutants lacking SOD activity are hypersensitive to damage by H_2O_2 (34), and extra SOD can often protect cells against damage by H_2O_2 , provided that it can enter the cell (58). These data are consistent with a role of $\text{O}_2^{\cdot-}$ in facilitating damage by H_2O_2 , and Equations 5 and 6 provide an explanation. However, many scientists are reluctant to believe that $\text{O}_2^{\cdot-}$ serves only as a reducing agent for metal ions since, in general, mammalian tissues are fairly reducing environments. The arguments have been rehearsed in detail (1,49,59) but the point is not yet settled.

Antioxidant Defenses

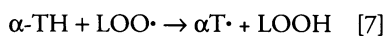
Living organisms have evolved antioxidant defenses to remove excess $\text{O}_2^{\cdot-}$ and H_2O_2 . Superoxide dismutases (SODs) remove $\text{O}_2^{\cdot-}$ by greatly accelerating its conversion to H_2O_2 (Equation 1). Human cells have a SOD enzyme containing manganese at its active site (Mn-SOD) in the mitochondria. A SOD with copper and zinc at the active site (Cu,Zn-SOD) is also present, but largely in the cytosol (5). Catalases in the peroxisomes convert H_2O_2 into water and O_2 and help dispose of H_2O_2 generated by peroxisomal oxidase enzymes (18).

However, the most important H_2O_2 -removing enzymes in human cells are glutathione peroxidases (GSHPX), which require selenium (as an active site selenocysteine residue) for their action. GSHPX enzymes remove H_2O_2 by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power (18). Another important antioxidant defense already referred to is the sequestration of transition metal ions into forms that will not catalyze free radical reactions (1,29,53). This is particularly important in the extracellular environment, where levels of SOD, GSH, GSHPX, and catalase are often very low (53).

Antioxidant defense enzymes are essential for healthy aerobic life. For example, SOD-negative mutants of *E. coli* will not grow aerobically unless given a rich growth medium, due to impaired biosynthesis of certain amino acids. Even when so supplemented, SOD *E. coli* cells grow slowly, suffer membrane damage, are abnormally sensitive to damage by H_2O_2 (perhaps because of Equations 5 and 6) and show a high mutation rate (34).

However, antioxidant defenses exist as a balanced and coordinated system. Thus, although SOD is important, an excess of SOD in relation to peroxide-metabolizing enzymes can be deleterious (60–63). This has been shown by transfecting cells with human cDNAs encoding SOD (60). Transgenic mice overexpressing human Cu,Zn-SOD are resistant to elevated O_2 and to certain toxic agents (62,63) but they show certain neuromuscular abnormalities resembling those found in patients with Down's syndrome (62). The gene encoding Cu,Zn-SOD is located on chromosome 21 in humans, and Down's syndrome is usually caused by trisomy of this gene, raising tissue Cu,Zn-SOD levels by about 50%. The limited data available at present are consistent with the view that the excess of Cu,Zn-SOD may contribute to at least some of the abnormalities in patients with Down's syndrome (62).

In addition to antioxidant defense enzymes, some low-molecular-mass free radical scavengers exist. Reduced glutathione can scavenge various free radicals directly, as well as being a substrate for GSHPX enzymes. α -Tocopherol is the most important free radical scavenger within membranes. Attack of reactive radicals, such as OH^{\cdot} , upon membranes can damage them by setting off a free radical chain reaction called lipid peroxidation. α -Tocopherol (α -TH) inhibits this by scavenging peroxy radicals (Table 1), intermediates in the chain reaction.



However, the tocopherol thereby becomes a radical, αT^{\cdot} . This illustrates a fundamental principle of free radical chemistry: when radicals react with nonradicals, new radicals are generated. Only when two radicals meet and join their unpaired electrons are the radicals lost (termination reactions). An example is the reaction of $\text{O}_2^{\cdot-}$ with NO^{\cdot} (Equation 3).

Overall, antioxidant defenses seem to be approximately in balance with generation of oxygen-derived species *in vivo*. There appears to be no great reserve of antioxidant defenses in mammals, perhaps because, as pointed out previously, some oxygen-derived species perform useful metabolic roles.

Oxidative Stress: A Definition

Generation of ROS and the activity of antioxidant defenses appear more or less balanced *in vivo*. In fact, the balance may be slightly tipped in favor of the ROS so

that there is continuous low-level oxidative damage in the human body. This creates a need for repair systems that can deal with oxidatively damaged molecules (Table 2). However, if a greater imbalance occurs in favor of the ROS, oxidative stress is said to result (19). Most aerobes can tolerate mild oxidative stress: indeed they often respond to it by inducing synthesis of extra antioxidant defenses. For example, if rats are gradually acclimatized to elevated O_2 , they can tolerate pure O_2 for much longer than naive rats, apparently due to increased synthesis of antioxidant defenses in the lung (64,65). Other examples are the complex adaptive response of *E. coli* treated with low concentrations of H_2O_2 (66) and the activation of NF-KB in oxidatively stressed mammalian cells (33).

However, severe oxidative stress can cause cell damage and death. In mammalian cells, oxidative stress appears to cause increases in the levels of free Ca^{2+} (67) and free iron (68) within cells, e.g., by damaging proteins that normally keep these metal ions safely bound. Iron ion release can lead to OH^{\cdot} generation, which has been shown to occur within the nucleus of H_2O_2 -treated cells (56). An excessive rise in intracellular free Ca^{2+} can also activate endonucleases and cause DNA fragmentation (67).

Hence, oxidative stress results in damage to DNA, proteins, lipids, and carbohydrates (67,68). The relative importance of damage to these different molecular targets in mediating cell injury or death depends upon what degree of oxidative stress occurs, by what mechanism it is imposed, for how long, and the nature of the system stressed. For example, lipid peroxidation appears to be an important consequence of oxidative stress in human atherosclerotic lesions (69). Several halogenated hydrocarbons (such as CCl_4 and bromobenzene) appear to exert some, or all, of their toxic effects by stimulating lipid peroxidation *in vivo* (7). However, for most other toxic agents causing oxidative stress, lipid peroxidation is not the major mechanism of primary cell injury: damage to proteins and DNA is usually more important (1,47,68). For example, it has often been assumed that lipids are a major target of damage by inhaled ozone, but proteins may be equally or more important (see below).

Causes of Oxidative Stress: Toxic Agents

Oxidative stress can be imposed in several ways. Thus, severe malnutrition can deprive humans of the minerals (e.g., Cu, Mn, Zn,

Table 3. Why cigarette smoke can impose oxidative stress.

- (1) Smoke contains many free radicals, especially peroxy radicals, that might attack biological molecules and deplete antioxidants, such as vitamin C and α -tocopherol.
- (2) Smoke contains oxides of nitrogen, including the unpleasant nitrogen dioxide (NO_2).
- (3) The tar phase of smoke contains hydroquinones. These are lipid-soluble and can redox-cycle to form $\text{O}_2^{\cdot-}$ and H_2O_2 . They can enter cells and may even reach the nucleus to cause oxidative DNA damage. Some hydroquinones may release iron from the iron-storage protein ferritin in lung cells and respiratory tract lining fluids.
- (4) Smoking may irritate lung macrophages, activating them to make $\text{O}_2^{\cdot-}$.^a
- (5) Smokers' lungs contain more neutrophils than the lungs of nonsmokers, and smoke might activate these cells to make $\text{O}_2^{\cdot-}$.^a
- (6) Smokers often eat poorly and drink more alcohol than nonsmokers and may have a low intake of nutrient antioxidants.

^a The effects of cigarette smoke on phagocytes are dose-related. Low levels may stimulate them, but high levels may poison them and so depress their activity.

Se) and vitamins (e.g., riboflavin—needed for the FAD cofactor of glutathione reductase, and α -tocopherol—needed for antioxidant defense) (1). More usually, however, the stress is due to production of excess ROS.

Several drugs and toxins impose oxidative stress during their metabolism. Carbon tetrachloride is one example (Table 1). Another is paraquat, a herbicide that causes lung damage in humans. Its metabolism within the lung leads to production of large amounts of $\text{O}_2^{\cdot-}$ and H_2O_2 (1). Gas-phase cigarette smoke also imposes some oxidative stress. Some of the reasons for this are summarized in Table 3 (70,71).

Causes of Oxidative Stress: Disease and Tissue Injury

Does oxidative damage play a role in human disease? Many of the biologic consequences of excess radiation exposure may be due to OH^{\cdot} -dependent damage to proteins, DNA, and lipids (6). Oxidative damage (resulting from exposure to elevated O_2 in incubators) may account for damage to the retina of the eye (retinopathy

of prematurity) in premature babies (3). However, there are many papers in the biomedical literature suggesting a role for oxidative stress in other human diseases [over 100 at the last count (1)].

Tissue damage by disease, trauma, poisons, and other causes usually lead to formation of increased amounts of putative "injury mediators," such as prostaglandins, leukotrienes, interleukins, interferons, and tumor necrosis factors (TNFs). All of these have at various times been suggested to play important roles in different human diseases. Currently, for example, there is much interest in the roles played by $\text{TNF}\alpha$, NO^{\cdot} , and interleukins in adult respiratory distress syndrome and septic shock (8,72). ROS can be placed in the same category, i.e., tissue damage will usually lead to increased ROS formation and oxidative stress. Figure 1 summarizes some of the reasons for this. Indeed, in most human diseases, oxidative stress is a secondary phenomenon, a consequence of the tissue injury. That does not mean it is not important (1,72). For example, excess production of $\text{O}_2^{\cdot-}$, H_2O_2 , and other species by phagocytes at sites of chronic inflammation can cause severe dam-

age. This seems to happen in the inflamed joints of patients with rheumatoid arthritis (72) and in the gut of patients with inflammatory bowel diseases (73). Tissue injury can release metal ions from their storage sites within cells, leading to OH^{\cdot} generation (72,74). Thus, the main question about ROS in human disease is not "can we demonstrate oxidative stress?" but rather "does the oxidative stress that occurs make a significant contribution to disease activity?" The answer to the latter question appears to be "yes" in at least some cases, including atherosclerosis, rheumatoid arthritis, and inflammatory bowel disease (1,69,72–74). However, it may well be "no" in many others. Elucidating the precise role played by free radicals has not been easy because they are difficult to measure, but the development of modern assay techniques is helping to solve this problem (72).

Causes of Oxidative Stress: Environmental Air Pollutants

The role of free radicals in the toxicity of O_2 and of cigarette smoke (Table 3) has already been discussed, but oxidative damage is frequently suggested to be involved in the deleterious effects of O_3 and NO_2 (75,76). O_3 is not a free radical, but it can oxidize many biological molecules directly and, in addition, it reacts slowly with water at alkaline pH to give OH^{\cdot} (77). It has also been suggested to produce singlet O_2 when it reacts with biological molecules (78).

The first biological fluids that come into contact with inhaled O_3 are the respiratory tract lining fluids (RTLFs), that presumably serve to absorb and detoxify some of the inhaled O_3 so as to lower the amount that enters the more vulnerable peripheral gas exchange regions of the lung. Some information is available about the antioxidants of these fluids (79,80) but the problems of sampling them (by the techniques of respiratory tract lavage) have hindered elucidation of their precise comparative antioxidant capabilities, since lavage itself produces considerable and variable dilution of RTLFs and some of their constituents may be oxidized during the procedures. In addition, the antioxidants present depend upon which part of the respiratory tract is being sampled (79), i.e., nasal passages, airways, bronchioles, alveoli. Often a mixed fluid is obtained.

By contrast, the antioxidant defenses of human plasma have been well characterized (53). To approach an understanding of how O_3 might interact with a complex biological fluid, the reactions of O_3 with freshly prepared human plasma have been studied.

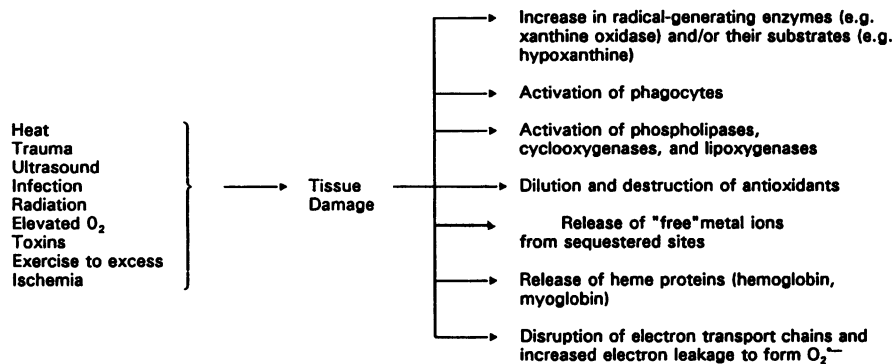
**Figure 1.** Some of the mechanisms by which tissue damage can cause oxidative stress.

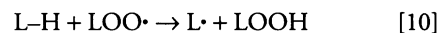
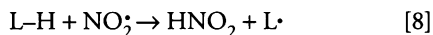
Table 4. A comparison of the effects of cigarette smoke (CS), O₃, and NO₂ on human plasma.

Event	CS	O ₃	NO ₂
Oxidation of ascorbate	++++	++++	++++
Breakdown of uric acid	++	++++	+++
Loss of plasma -SH groups	+++	+++	+++
Formation of protein carbonyls	++++	++	○
Depletion of α-tocopherol	++	○	++
Lipid peroxidation	+	○	++

Indeed, oxidant injury to the lung causes increased influx of plasma constituents, so that the lung lining fluids become more like plasma in composition. Uric acid and ascorbic acid were found to be the major plasma scavengers of O₃ (81). Although it is often assumed that lipids are a major target of attack by O₃, no evidence of substantial lipid damage by O₃ was obtained (81), in keeping with other studies in the literature (82,83). Instead, oxidative protein damage was observed in O₃-exposed plasma, as -SH group loss and protein carbonyl formation (84). However, it will be necessary to study human lung lining fluids to substantiate this conclusion, especially as Cueto et al. (85) found end products of lipid damage in lung lining fluids from rats after O₃ exposure.

Since uric acid is a major constituent of upper airway lining fluid in humans (79), it could act as a "scrubber," decreasing the concentration of O₃ in inhaled air so as to protect the more vulnerable alveolar regions of the lung (81).

By contrast with the effect of O₃, NO₂ did not generate protein carbonyls in plasma, although nitration of aromatic amino acids took place and protein -SH groups were lost, presumably by direct reaction with NO₂ (86). Again unlike O₃, NO₂ induced lipid peroxidation in plasma, presumably by the reactions



Initiation of peroxidation by NO₂ (Equation 8) presumably sets up the autocatalytic chain reaction of lipid peroxidation (Equations 9,10) resulting in the accumulation of lipid peroxides (LOOH). Uric acid and ascorbate were found to be important antioxidants protecting plasma against oxidative damage by NO₂, but the lipid-soluble antioxidants α-tocopherol and ubiquinol probably also play an important protective role in limiting lipid peroxidation (86).

Hence, as summarized in Table 4, O₃ and NO₂ damage different molecular targets, which could be one reason why they might sometimes exert synergistic damaging effects *in vivo*. For comparison, Table 4 also includes the effects of cigarette smoke upon plasma. Ascorbic acid is rapidly oxidized and damage to both proteins (carbonyl formation, loss of -SH groups) and lipids (lipid peroxide formation) occurs. Ascorbic acid protects the plasma against lipid damage by ozone, but does not protect against protein damage (87,88).

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